REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
22-04-2011	Final Report	1-Jun-2004 - 31-May-2009

4. TITLE AND SUBTITLE
Bio-Mechanical Interfaces for Cell-Based Microsystems

5a. CONTRACT NUMBER
W911NF-04-1-0171

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611103

6. AUTHORS
Chad Mirkin, Aaron Dinner, Michael Roukes, Anthony Evans, Robert McMeeking, Christopher Chen, Milan Mrksich

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Chicago
University Research Administration
970 East 58th Street
Chicago, IL 60637 -

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
46645-EG-MUR.1

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
The MURI represents a multidisciplinary program to develop hybrid microsystems that combine mammalian cells as functional components with conventional solid-state materials. Nanomechanical elements provide the interface between the adherent cells and materials, and are designed to transduce biological and mechanical signals at this interface. The first aim of the work has developed an integrated platform for installing mechanical interfaces to cells, including mechanical sensor and actuator arrays, surface chemistries to install selective interactions between

15. SUBJECT TERMS
biomechanics, force sensors, cell adhesion, cytoskeleton, surface chemistry, nanofabrication

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Milan Mrksich
UU	UU	UU	UU		19b. TELEPHONE NUMBER: 773-702-1651

Report Title

Bio-Mechanical Interfaces for Cell-Based Microsystems

ABSTRACT

The MURI represents a multidisciplinary program to develop hybrid microsystems that combine mammalian cells as functional components with conventional solid-state materials. Nanomechanical elements provide the interface between the adherent cells and materials, and are designed to transduce biological and mechanical signals at this interface. The first aim of the work has developed an integrated platform for installing mechanical interfaces to cells, including mechanical sensor and actuator arrays, surface chemistries to install selective interactions between the solid-state and cellular components, integration of engineered cells on the devices, development of multi-scale models to describe the bio-mechanical coupling, and characterization of the durability of cell-mechanical interfaces. The second aim employs this platform for fundamental studies to understand the basis for mechanical coupling including the mechanical signatures for several important cellular activities, the limits by which mechanical actuation can influence cellular behaviors and provides experimental data to guide model development and characterization of fatigue and failure in the devices. The third aim will apply these advances to prototype cell-based devices with high impact for the DoD. These microsystem technologies include stand-alone sensors for CBW agents, actuation in MEMS devices, active coatings for signature reduction, and hybrid technologies for extracting energy from the environment.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

1. Legant W.R., Pathak A., Yang M.T., Deshpande V.S., McMeeking R.M., Chen, C.S. "Microfabricated tissue gauges to measure and manipulate forces from 3D microtissues," Proc Nat Acad Sci 2009, 106, 10097-10102.

2. McGarry J.P., Fu J., Yang M.T., Chen C.S., McMeeking R.M., Evans A.G., Deshpande V.S. "Simulation of the contractile response of cells on an array of micro-posts," Philos. Transact. A Math. Phys. Eng. Sci. 2009, 367, 3477-97.

3. Yang, M.T., Chen, C.S. "Mechanotransduction and the Study of Cellular Forces," Methods in Bioengineering: Microdevices in Biology and Medicine. Artech House. 2009, 4, 63-86.

4. Sniadecki, N.J., Legant, W.R., Chen, C.S. "Micro- and Nanoscale Force Techniques for Mechanotransduction. Cellular Mechanotransduction: Diverse Perspectives from Molecules to Tissues," Cambridge University Press. 2009, 377-402.

5. Treiser, M.D., Yang, E.H., Gordonnov, S., Cohen, D.M., Androulakis, I.P., Kohn, J., Chen, C.S., Moghe, P.V. "Cytoskeleton-based Forecasting of Stem Cell Lineage Fates," Proc Nat Acad Sci. 2010, Epub ahead of Print.

6. Liu, Z., Sniadecki, N.J., Chen, C.S. "Mechanical Forces in Endothelial Cells during Firm Adhesion and Early Transmigration of Human Monocytes," Cellular and Molecular Engineering. 2010, Epub ahead of Print.

7. Li, Ying, Bhimalapuram, P. and A.R. Dinner. "Model for how retrograde actin flow regulates adhesion traction stresses," J Physics Condensed Matter. 2010, 22, 194113.

8. J.P. McGarry, J. Fu, M.T. Yang, C.S. Chen, R.M. McMeeking, A.G. Evans and V.S. Deshpande. "Simulation of Contractile Response of Cells on an Array of Micro-Posts," Philosophical Transactions of the Royal Society of London 2009, 367, 3477-3497.

9. W.R. Legant, A. Pathak, V.S. Deshpande, R.M. McMeeking, C.S. Chen. "Microfabricated Tissue Gauges to Measure and Manipulate Cellular Forces in 3D Tissues," Proceedings of the National Academy of Sciences 2009, 106, 10097-10102.

10. Zheng, Z. J.; Daniel, W. L.; Giam, L. R.; Huo, F.; Senesi. A. J.; Zheng, G.; Mirkin, C. A. "Multiplexed Protein Arrays Enabled by Polymer Pen Lithography: Addressing the Inking Challenge," Angew. Chem Int. Ed., 2009, 48, 7626-7629.

11. Senesi, A. J.; Rozkiewicz, D. I.; Reinhoudt, D. N.; Mirkin, C. A. "Agarose-Assisted Dip-Pen Nanolithography of Oligonucleotides and Proteins," ACS Nano 2009, 3, 2394-2402.

12. Huang, L.; Braunschweig, A. B.; Shim, W.; Qin, L.; Lim, J.-L.; Hurst, S.; Huo, F.; Xue, C.; Jang, J.-W.; Mirkin, C. A. "Matrix-Assisted Dip-Pen Nanolithography (MA-DPN) and Polymer Pen Lithography (MA-PPL)," Small 2010, 6, 1077-1081.

13. Liao, X.; Braunschweig, A. B.; Zheng, Z.; Mirkin, C. A. "Force- and Time-Dependent Feature Size Control on Molecular Printing via Polymer Pen Lithography (PPL)," Small 2010,6, 1082-1086.

14. Liao, X.; Braunschweig A. B.; Mirkin, C. A. ""Force-Feedback" Leveling of massively parallel arrays in polymer pen lithography," Nano Letters, 2009, 10, 1335-1340.

15. J.L. Eisenberg, J.L. Piper and M. Mrksich. "Using Self-Assembled Monolayers to Model Cell Adhesion to the 9th and 10th Type III Domains of Fibronectin," Langmuir, 2009, 25, 13942-13951.

Number of Papers published in peer-reviewed journals: 15.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Chad Mirkin
1.DARPA – MEMS PI Meeting, SunRiver, OR; “Scanning Probe Epitaxy,” (2009)
2.238th ACS National Meeting, Washington, D.C.; "Nanostructures in Medicine and Biology" and "Nanostructures as Labels and Taggants in High Sensitivity Detection and Encoding Systems," (2009).
3.Bio-X Taggant Review, Dayton, OH; “Plasmonic Encoding,” (2009).
4.6th Key Symposium on Nanomedicine, Stockholm, Sweden; "Polyvalent Nucleic Acid Nanoconjugates in Materials Synthesis, Biodiagnostics, and Intracellular Gene Regulation," (2009).
5.NIH Director’s Pioneer Award Symposium, Bethesda, MD; “Nanostructures for Problems in Biology,” (2009).
6.Micro-and NanoEngineering Conference, Brussels, Belgium, “Unconventional Approaches to Nanofabrication,” (2009).
7.Havinga Medal, Leiden University, Amsterdam, The Netherlands; “Programming the Assembly of Inorganic Materials with DNA: Applications in Biology and Medicine,” (2009).
8.NCI Nanotechnology Alliance Investigators Meeting, Manhattan Beach, CA; “Role of PCAST in Science,” (2009).
9.UOP-Honeywell, Des Plaines, IL; “Unconventional Approaches to Nanofabrication,” (2009).
10.Society of Analytical Chemists of Pittsburgh, Pittsburgh, PA; “Nanostructures in Biology and Medicine,” (2009).
11.University of Pittsburgh, PA; “Anistropic Nanostructures: Building Valency into Nanoparticles,” (2009).
12.Grand Valley State University, Allendale, MI; “Building Valency in Metallic Nanostructures: The Atom Analogy” and “Programming Materials Assembly with DNA: Applications in Biology and Medicine,” (2009).
13.Chemical and Biological Defense Science and Technology Conference, Dallas, TX; “Nanostructures in Biology and Medicine,” (2009).
14.Materials Research Society, Boston, MA; “Massively Parallel Polymer Pen Lithography,” “Rationally Designed Nanostructures Fabricated by On-Wire Lithography,” and “The Polyvalent Gold Nanoparticle Conjugate: Materials Synthesis, Biodiagnostics, and Intracellular Gene Regulation,” (2009).
15.NSF Nanoscale Science and Engineering Grantees Conference, Arlington, VA; “A PCAST Perspective on Nanotechnology Development,” (2009).
16.Ohio State University, Columbus, OH; “Programming Materials Synthesis with DNA: Applications in Biology and Medicine,” and “Unconventional Approaches to Nanofabrication,” (2010).
17.Pittcon Conference, Orlando, FL; “Multiplexed Detection with Nanodisk Codes,” and “Nanoflares: A New Modality in Biodiagnostics and Bioimaging,” (2010).
18.237th ACS National Meeting, San Francisco, CA; “Nanoflares for the Intracellular Detection of Small Molecules, Nucleic Acids, Metal Ions, and Protein Targets,” and “Anisotropic Nobel Metal Nanostructures,” (2010).
19.University of California – Berkeley, Berkeley, CA; “Programming the Assembly of Nanoparticles with DNA: The Atom Analogy,” (2010).
20.Herman S. Bloch Memorial Lecture, University of Chicago, Chicago, IL; “Molecular Printing: A Chemist's Approach to Desk Top Fab,” (2010).
21.101st American Association for Cancer Research (AACR) Annual Meeting, Washington, DC; “The Polyvalient Nanoparticle Conjugate: A New Frontier in Cancer Diagnostics and Therapeutics,” (2010).
22.James Madison University, Harrisonburg, VA; “Nanotechnology: Small Wonders, Medical Miracles,” (2010).
23.University of Kentucky, Lexington, KY; “Programming Materials Synthesis with DNA: Applications in Biology and Medicine,” (2010).
24.Digestive Disease Week for Society for Surgery of the Alimentary Tract, New Orleans, LA; “Nanotechnology in Biology and Medicine,” (2010).

Robert McMeeking.

25. Composites at Lake Louise 2009, Lake Louise, Alberta, Canada; “Models for the contractile formation of composite tissue constructs,” October 25-30, 2009.
26.Applied Mechanics Seminar, Caltech; “The mechanics of the cytoskeleton and cell adhesions,” November 6, 2009.
27.Mechanical Engineering Seminar, Northwestern University; “The mechanics of the cytoskeleton and cell adhesions,” January 26, 2010.
28.Materials Research Lab Macromolecules Thrust Seminar, UCSB; “The mechanics of the cytoskeleton and cell adhesions,” June 2, 2010.

Milan Mrksich
29.Glasgow Symposium - United Kingdom, Scotland; "Model Surfaces for Studying Cell Adhesion" (2009).
30.Georgia Institute of Technology Colloquium: Molecular Science and Engineering, Atlanta, GA; "Tailored Substrates for Cell Biology," (2009).
31.HHMI Scientific Meeting - Chevy Chase, MD; "Model Substrates for Studying Adhesion Receptors: Revising the Platelet Integrin," (2009).
32.PSD/BSD Fall Visiting Committee Meeting, Chicago, IL; "Joining Cells and Materials and Programming Biological Systems to guide their behavior," (2009).
33.Cell and Molecular Biology Seminar, Northwestern University, Evanston, IL; "Model Substrates for Studying Cell Adhesion," (2009).
34.Chicago Insights Atlanta, Atlanta, GA; "Writing the Blueprint for Life," (2010).
35.237th ACS National Meeting, San Francisco, CA; "Tailored Substrates for Studying and Controlling Cell Adhesion," (2010)

Number of Presentations: 35.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

1. Chai, J.; Huo, F.; Zheng, Z.; Giam, L. R.; Shim, W.; Mirkin, C. A. “Scanning Probe Block Copolymer Lithography,” Science, 2010, submitted.

2. Huo, F.; Zheng, G.; Liao, X.; Giam, L. R.; Chai, J.; Chen, X.; Shim, W.; Mirkin, C. A. “Beam Pen Lithography”, Nature Nanotechnology, 2010, submitted.

3. Chai, J.; Huo, F.; Zheng, Z.; Giam, L. R.; Shim, W.; Mirkin, C. A. "Scanning Probe Block Copolymer Lithography," Science, 2010, submitted.

4. A.Pathak, R.M. McMeeking, A.G. Evans and V.S. Deshpande. "An Analysis of the Co-operative Mechano-Sensitive Feedback between Intercellular Signaling, Focal Adhesion Development and Stress Fiber Contractility,” submitted to Journal of Applied Mechanics (2009).

Number of Manuscripts: 4.00

Patents Submitted

NU 29194, "Massively Parallel Lithography with Two-Dimensional Pen Array" CA Mirkin, P Sun, Y Wang, S Lenhert. ID Submitted 11/20/2009

Incorporated into US regular patent application No. 11/690,738 filed on 3/23/2007, which had been incorporated into US provisional application serial no. 60/792,950.

Patents Awarded

Awards

1. 2010 Elected Member of the National Academy of Sciences, C. Mirkin.
2. 2010 Herman S. Bloch Award for Scientific Excellence in Industry, University of Chicago, C. Mirkin.
3. 2010 Einstein Professorship of the Chinese Academy of Sciences (CAS), C. Mirkin
4. 2010 Ohio State University Edward Mack Jr. Memorial Award, C. Mirkin

Graduate Students

NAME	PERCENT_SUPPORTED
Daniel Eichelsdoerfer	1.00
Amit Pathak	0.50
James Kornacki	0.02
Jessica Eisenberg	1.00
Shuheng Li	0.88
Juan Sanchez Cortez	1.00
Ying Li	0.38
FTE Equivalent:	**4.78**
Total Number:	**7**

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Kristin Beaumont	0.88
FTE Equivalent:	**0.88**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Amit Pathak
Jessica Eisenberg
Total Number: **2**

Names of other research staff

NAME	PERCENT SUPPORTED	
Louise Giam	0.00	No
FTE Equivalent:	**0.00**	
Total Number:	**1**	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

This Report summarizes progress in MURI program on "Bio-Mechanical Interfaces for Cell-Based Microsystems" (Agreement Number W911NF-04-1-0171). The MURI has had the objective of developing the tools that enable an understanding of the role of mechanics in cell function and that enable the engineering of cells that can be mechanically coupled to engineered platforms for a variety of purposes. The project has had several significant accomplishments in several areas, including the fabrication of mechanical sensor/actuator arrays, integration of cells with materials, development of models for understanding and describing the intersection of mechanics and cell function, and the use of mechanics to read and direct cell function. The present report summarizes much of the key accomplishments during this period but is not exhaustive in its coverage.

Solid-State Mechanical Sensor Arrays for Cellular Transduction

We have developed a tool, the Single-Cell-Pico-Force-Microscopy (SCPFM), capable of measuring cellular forces from individual adherent cells with the high resolution necessary to track macro-molecular force dynamics in living cells. Existing tools capable of measuring cell forces, while varied, are all based on optical detection and are limited in sensitivity, range or the physiological relevance of their coupling to the cytoskeleton. SCPFM overcomes these limitations by utilizing novel polymer Nano-Electro-Mechanical-Systems (NEMS) force sensors. SCPFM is a fully integrated, monolithic instrument that combines the near-single molecule force measurement capabilities of (NEMS) based force sensors with integrated microfluidics that enable precision fluid control, and thereby precision pharmacological stimulation of individual cells. SCPFM enables for the first time the systematic study of the molecular-mechanical responses of individual cells to pharmacological and mechanical stimulation.

We have developed novel polymer NEMS devices that contain integrated metallic piezoresistive (strain coupled) force sensors. Polymer (SU-8, polyimide or parylene) is used because the low Young's modulus enables improved sensitivity and compliance matching to typical biological materials. The fabrication process is complex but wafer scale processing with high yields (>70%) is routine. The devices are abundant and disposable. We employ a multilayer microfluidic cassette to encapsulate the NEMS force sensors. A glass cover slip seals the backside of the chip and enables *in vivo* high-resolution optical microscopy. The workstation, shown in Figure 1, integrates all of these technologies into a single complete system with fluorescence microscopy, simultaneous fluidic operations, and high resolution force measurement for studying the cell-mechanical interface.

We have demonstrated the capabilities of SCPFM by perturbing adherent NIH-3T3 cells with cytochalasin D. We observe the expected force collapse upon cytochalasin D exposure and recovery when the drug is removed at very similar magnitudes and

time scales as has been seen with optical methods. The fine structure of the force vs time data illustrates the immense potential of SCPFM. The data show force signatures of two previously inaccessible molecular-mechanical processes.

Figure 1. Left, NEMS chip encapsulated in microfluidics. The microfluidics include 40 computer operated control lines, 15 fluid input/output lines and one gas inlet line all built into an 80mm^2 footprint. The microfluidics are critical for separating the electronics from the fluids – controlling stray capacitances in particular, – enabling controlled media perfusion for long term tissue culture, controlling placement of a cell on the force sensor and precise pharmacological perturbation of the cell. Center and right, the microscope-mounted-incubator-and-sample-holder includes freedthrus for 64 microfluidic lines, 40 electrical lines, CO2 sensor, temperature

Elastomeric Mechanical Transducer Arrays

Early in the program we had demonstrated the ability to measure cellular traction forces using a microfabricated array of discrete, deformable PDMS cantilevers, or mPADs (microfabricated post array detectors). We have built upon this approach for the purposes of better understanding single cell mechanics, and addressed several efforts: (1) To further develop image analysis algorithms that increase force measurement sensitivity of post array devices; (2) To establish approaches to apply forces to cells while simultaneously measuring contractile forces in the cells; (3) To study the relationship between the adhesions formed at the cell-substrate interface and the forces experienced at those adhesions; (4) To develop a higher density post array device, that when coupled with more robust image analysis software, will allow greater sensitivity for force measurements; (5) To study the spatiotemporal relationship between actomyosin stress fiber contraction and the traction forces observed on the mPADs.

We have developed a device that can be shared between investigators. In our original conception, positions of the tips of the posts were determined by acquiring an image of the tips of the posts, and then manually aligning an ideal grid to the images in order to extract centroid coordinates for each post. This method not only assumes that the microfabricated post array is a highly precise, regular array, but allows for user bias and is time intensive. We previously reported in year one of the MURI a higher throughput, more robust method. In this new method, the entire surface of the mPAD posts is imaged, using the base of the posts as the undeflected position, and the tips as the deflected position. Post deflections are determined by using a binary image

thresholding algorithm to determine the centroid of each post at the tip and base. This method has been implemented in a semi-automated code for fixed cell image analysis, as well as a fully-automated code for high-volume image processing, especially for images taken in live cell, time-lapse experiments. To further improve our image analysis methods, this past year we have developed a more accurate centroid-positioning method, in which a 2D Gaussian is fit to the intensity profile of the pixels that make up each post in an image. This advancement increases the robustness and accuracy of our post deflection measurements by 1-2 orders of magnitude. We have also adapted the Gaussian centroid-determination method to measure forces in magnetically actuated mPADs and high-density nPADs (nanoposts).

In order to build active force probes into our mPAD device, we have incorporated ferromagnetic nanowires (from Prof. Daniel Reich, Johns Hopkins University) into the posts as actuators. Our current devices use Ni or Co nanowires electrochemically grown with 350 nm diameters and 5-10 micron lengths that can be embedded into PDMS posts. By applying a uniform magnetic field, B, perpendicular to the magnetic moment of the nanowire, μ, we can impart a torque, $\tau = \mu \times B$, that causes the post to bend in the direction of the field (Figure 2A). Ferromagnetic nanowires do not interfere with the curing chemistry of PDMS and are easily integrated into the PDMS (Figure 2B). Moreover, we have demonstrated that piezomagnetic actuation can be achieved. We conducted experiments with electromagnetic coils (B = ± 0.2T) acting on the posts. The posts bend in direct proportion to the applied field and we measured maximum deflections to be δ = ±0.25 μm (Figure 2C,D).

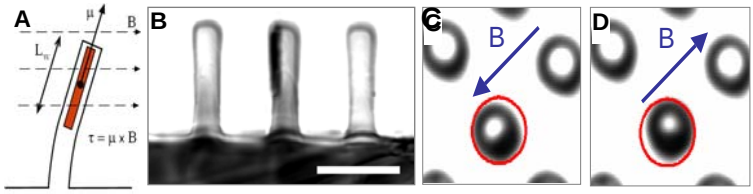


Figure 2. Schematic of piezomagnetic torque of a nanowire in a PDMS post (A). Cross-sectional optical micrograph showing a nanowire embedded in a PDMS post (B, bar: 10 μm). Image frames taken from a video show magnetic actuation of a nanowire post at (C) B = -0.2 T. (D) B = +0.2T. Red circles are

Despite this shortcoming, we have been able to examine mechanotransduction of fibroblasts cultured on the posts with nanowires. On an inverted fluorescent microscope, we recorded the change in post deflections of an NIH 3T3 cell (outlined with black trace) with respect to the first video frame (t = 0') (Figure 3A). When the magnetic field (B = 90mT) was turned on (after t = 10'), the nanowire post (red circle) was immediately observed to deflect in the field's direction. Subsequently, the fibroblast changed its contractile forces on the posts globally as a result of the mechanical stimulation (Figure 3B). This change is reported by the root mean square (rms) of the displacements, $\Delta r_{rms} = \sqrt{\frac{1}{N}\sum_i (\mathbf{r}_i - \mathbf{r}_{0,i})^2}$, where $\mathbf{r}_{0\,i}$ is the initial position of each post, summed over the posts under the cell (Fig. 3C). We find causality to exist between the nanowire actuation and change in cellular forces because the fibroblast

had low mechanical activity prior to the stimulation, but afterwards, it changed its cellular forces on posts relative to the first frame. To further examine the traction force dynamics of fibroblasts cultured in response to small externally applied forces, we are using a Gaussian centroid-determination method capable of detecting more subtle changes in post deflections.

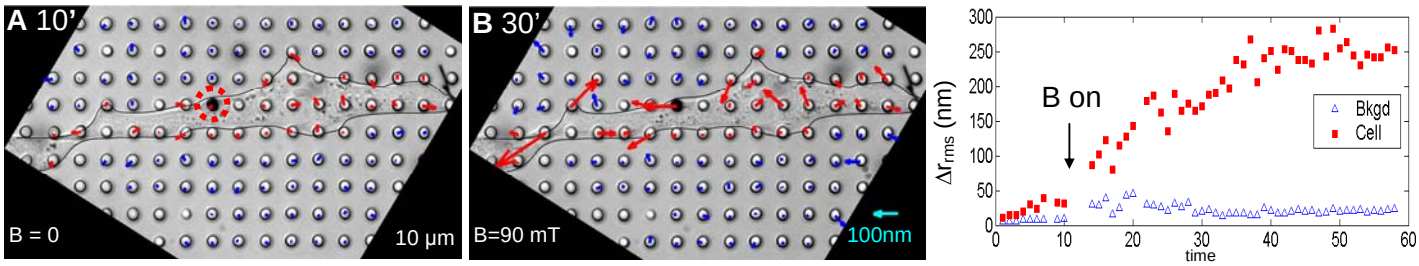


Figure 3. Changes in cellular forces measured (A) before and (B) 20 min after stimulation with a 90 mT field applied to a magnetic post (red circle in A). Arrows at posts show deflections from original positions at t = 0 for posts under cell (red) and posts in the background (blue). (C) Root mean square of the magnitude of deflection per post, r_{rms}, in contact

In addition to using "magnetic" mPADs to investigate traction force dynamics, we have also studied the effect of external force on focal adhesion dynamics. We find that force stimulation causes an increase in focal adhesion area at the site of stimulation, while distal adhesions do not increase in area (Figure 4). These results indicate that integrating magnetic materials into the mPADS is possible for the realization of an active microdevice and that cells respond to the mechanical stimulation imparted on them from the nanowires in the posts.

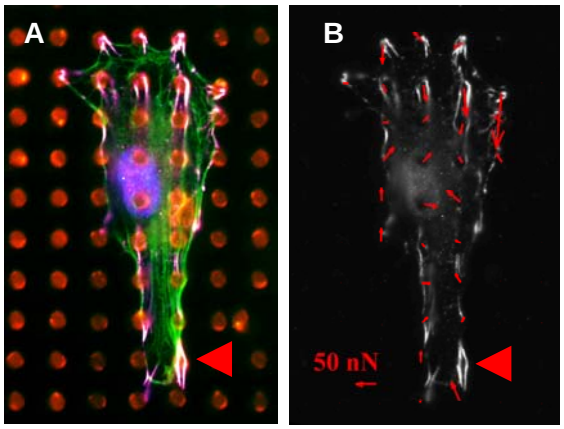


Figure 4. Representative image of a 3T3 Fibroblast stimulated with external force locally (indicated by red arrowhead), with an increase in focal adhesion area at the

We have also developed a higher density post array as a next-generation platform for traction force measurement, heretoforth referred to as the nPADs. Multiple arrays with different geometries were fabricated by MEMS exchange, allowing us to examine cellular traction forces on posts with different diameters (500 nm-1.25μm), heights (1.8-3.6 μm), and spacings (1.5-4.5 μm) (Figure 5A). Our devices are estimated to have post

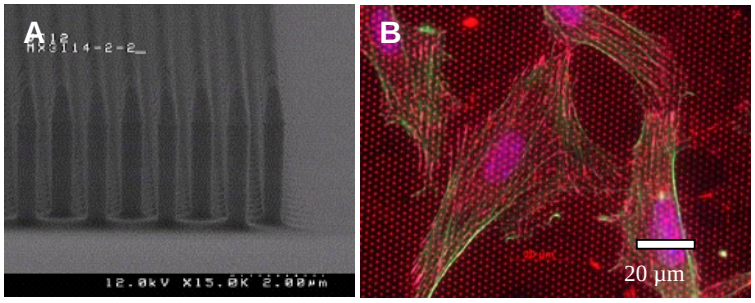


Figure 5. SEM image of a silicon nanopost array (A). Cells were plated on
PDMS nanoposts which were printed with a mixture of fibronectin and Alexa

stiffnesses ranging from 0.5-150 nN/μm^2. To analyze the deflections of hundreds of posts per cell, we are adapting the Gaussian centroid-determination algorithm for use on the nPADs. Concurrently, we are testing different methods to visualize the posts so as to minimize artifacts (i.e. non-specific fluorescence, cell membrane staining, opaque cell organelles) that would interfere with post centroid determination (Figure 5B). These methods include staining the posts with different fluorescent molecules or imaging the posts with bright-field transmission light. The ultimate goal is to develop a fully automated code and methodology for measuring a large number of post deflections with great accuracy and speed. The generality of our code design means that future translation of this approach to the mPADs for analysis of single cells at high magnification, and monolayers at low magnification is likely.

We have identified several critical questions regarding the mechanics of cells. One of these questions is to what extent the actin cytoskeleton and stress fibers contribute to the mechanical forces generated by cells. As described below, we have modeled the actin cytoskeleton as a discrete network of stress fibers connected to posts (Figure 6). We have also developed continuum models of general cell contractility and the role of cell shape and substrate compliance in observed traction force distributions. We have found that the passive contribution to cellular mechanics is minimal compared to the ATP-dependent, myosin-driven contractile forces (Figure 7). This represents a key finding that has never been explored or modeled in the context of single cells.

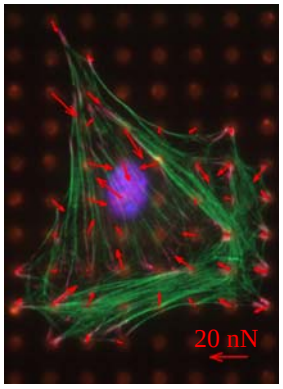


Figure 6. Actin fiber network and traction force map of a 3T3 fibroblast on the

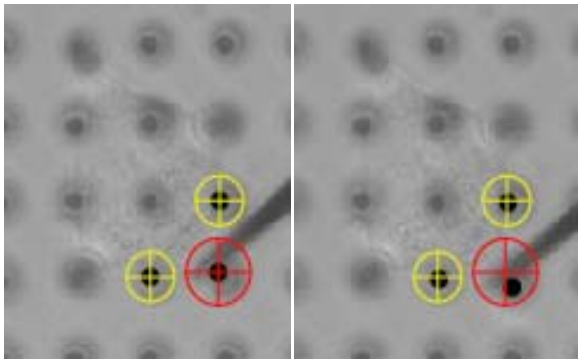


Figure 7. Images of a cell on mPADs before (left) and during (right) micromanipulation. The post in the red reticle is pulled. Images shown are taken 4 seconds apart. There is subtle pulling of neighboring posts (in yellow

Surface Patterning and Functionalization

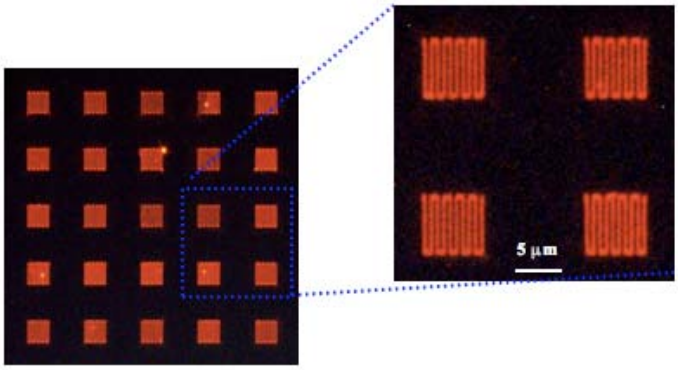


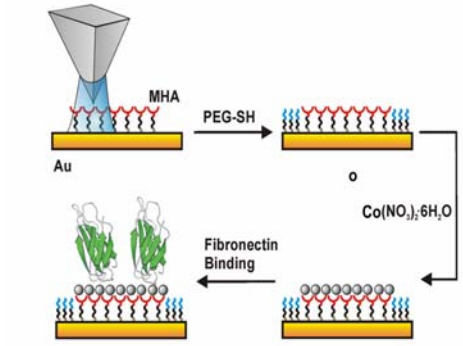
Figure 8. Fluorescence microscopy images of nanoarrays of phospholipid doped with 1 mol% rodamine-labeled DOPC generated by a 55,000 2D array.

We have made substantial progress in the development of patterning methods and surface chemistries for controlling the biological properties of planar surfaces. Dip-Pen Nanolithography (DPN) is a scanning probe-based technique

that uses an ink-coated atomic force microscope (AFM) tip to pattern surfaces on the sub-micron length scale. We have successfully demonstrated that DPN can be used to generate nanoarrays of proteins that are relevant to controlling cell interactions with substrates. We have also extended the use of 2D arrays to the nanopatterning of biological lipids. Nanoarrays of biological lipids can be considered a model for cell surfaces as the components within a lipid nanoarray can be individually addressed. Biological lipid nanoarrays also allow one to investigate biological processes ranging from simple ligand/receptor interactions to cell-cell signaling events. Figure 8 displays the fluorescence microscopy images of nanoarrays of phospholipid doped with 1 mol% rhodamine-labeled DOPC generated by a 55,000 2D array.

2D arrays are currently limited to the deposition of only one type of ink, and the fabrication of multi-component nanoarrays still represents a challenge. We are currently developing novel strategies for the direct writing of multi-component biomolecules in a parallel fashion. Preliminary results indicate that one can use inkjet printing technology to ink individual tips with different ink molecules. In addition, efficient methodologies such as matrix-assisted nanopatterning, for the direct writing of biomolecules by DPN without the need for additional tip modification are being developed. Having a massive array of biologically active structures will allow one to move forward and probe cell-surface systems with high-resolution and registration, perhaps even at the single biomolecule level.

We have applied this patterning tool to control the mechanical linkages between a cell and its substrates. The approach uses substrates that are patterned with features that control the sizes of the focal adhesions that integrate the mechanical cytoskeleton with the substrate. By controlling the size of the focal adhesion, we control the mechanical load exert by the cell on the substrate. We fabricated fibronectin nanoarrays by immobilization of the protein on 16-mercaptohexadecanoic acid (MHA) affinity templates through coordination chemistry (Figure 9).



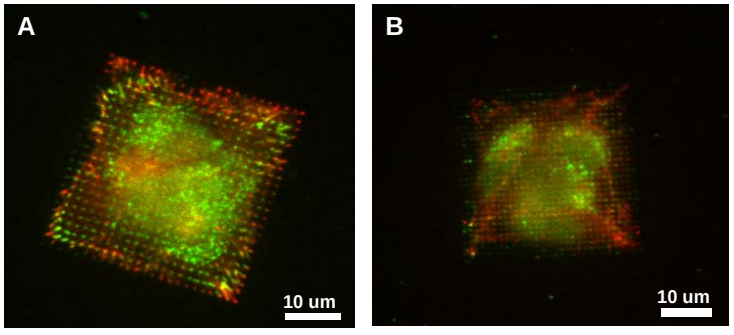


Figure 10. Representative fluorescence microscope images of high density protein nanoarrays immobilized through Co^{2+} affinity templates, and their interaction with H1080 cells. **(A)** and **(B)** An overlay image of the green and red channels, depicting a correlation between focal adhesion sites (red) with fibronectin arrayed onto the surface (green). **(A)** The diameter is 500 nm and the ligand spacing 1 um. **(B)** The diameter is 300 nm and the ligand spacing 600um.

When an eGFP-paxillin transfected cell line was used for these experiments, we could observe the formation of focal adhesion contacts in real-time using fluorescence microscopy. This approach provides many new opportunities to study focal adhesion formation, from monitoring the kinetics of focal adhesion formation to

observing the rearrangement of the actin filament structures in response to clustering of the integrin receptor proteins. Our experiments showed that the ability of a cell to form adhesion contacts is dependent on the spacing of the fibronectin proteins on the surface (Figure 10). Cells did not form adhesion contacts when the spacing was larger than 1.2 μm, and in most cases, they could not be found on the nanopatterned arrays.

These tips were able to generate patterns in a multiplexed fashion. We plan to optimize the conditions for homogeneous delivery of various proteins while increasing the number of ink materials that can be delivered to the same pattern. These large area nanopatterned arrays will then be used for cell studies to understand how focal adhesion complexes form or change in the context of size and protein presentation.

Finally, we have also investigated the impact on FAs of features other than applied force. These include the affinity of integrins (Figure 11) for extracellular ligands, integrin trafficking, membrane fluctuations, and retrograde actin flow. Because the protein receptor-ligand interactions of interest are confined to interfaces, we have additionally studied the effects on protein dynamics of limiting reactions to surfaces and polymers. These studies complement those focused on applied force and contractility and are important for the development of quantitative structure-property relationships that could be used as readouts for cell-based nanomechanical sensors based on FAs.

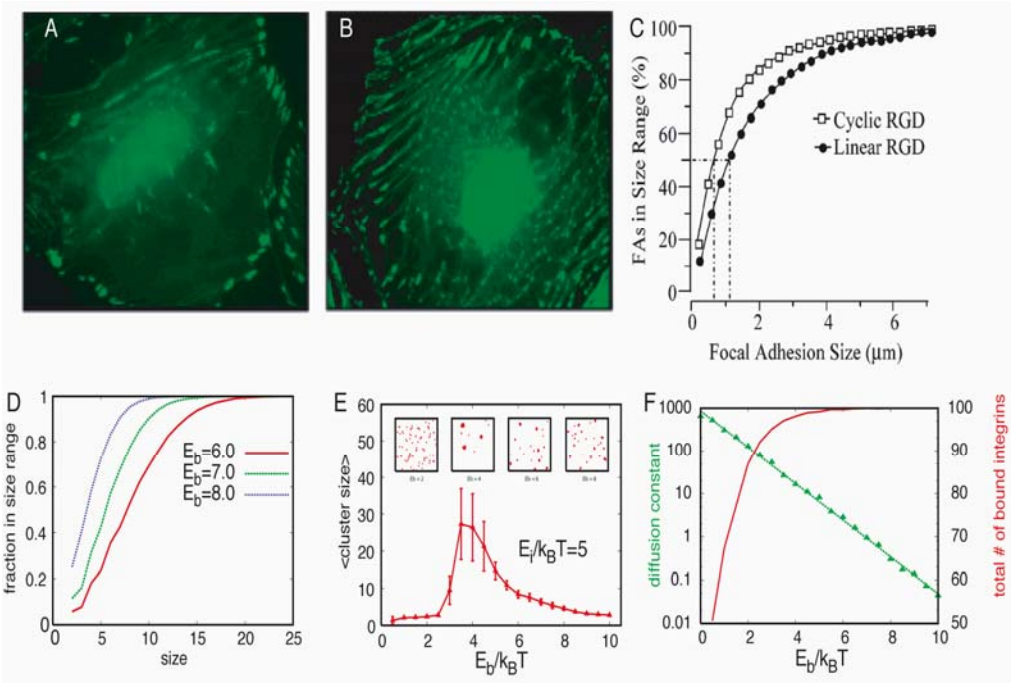


Figure 11: How integrin-substrate affinity impacts FA size. Examples of cells on surfaces presenting **(A)** low-affinity linear and **(B)** high-affinity cyclic RGD peptide. Observed **(C)** and calculated **(D)** cumulative histograms of FA sizes; higher E_b is stickier. A non-monotonic dependence of FA size on affinity over larger affinity ranges **(E)** revealed a competition between kinetic and thermodynamic factors **(F)**. Experimental data are from Kato and Mrksich (2004).

Modeling Bio/Mechanical Effects

A major theme of the project was the development of models of the cytoskeleton that account for the relationship of the mechanical properties of the cell and the molecular interactions that join the cell to a solid substrate. A summary of this work is shown in Figure 12. In addition, simulations were carried out for microtissue constructs, demonstrating that the models developed for a single cell can be extended to represent aggregates of cells. In parallel experiments carried out at the University of Pennsylvania, microcantilevers were used to simultaneously constrain the remodeling of a collagen gel and to report forces generated during this process. Independently varying the mechanical stiffness of the cantilevers and collagen matrix revealed that cellular contractile forces increased with boundary or matrix rigidity while levels of cytoskeletal and extracellular matrix (ECM) proteins correlated with levels of mechanical stress. By mapping these relationships among cellular and matrix mechanics, cellular contractile forces, and protein expression onto the bio-chemo-mechanical model of microtissue contractility, it was demonstrated how intratissue gradients of mechanical stress can emerge from collective cellular contractility and finally, how such gradients can be used to engineer protein composition and organization within a 3D tissue. Together, these findings highlight a complex and dynamic relationship between cellular forces, ECM remodeling, and cellular phenotype and describe a novel system to study and apply this relationship within engineered 3D microtissues.

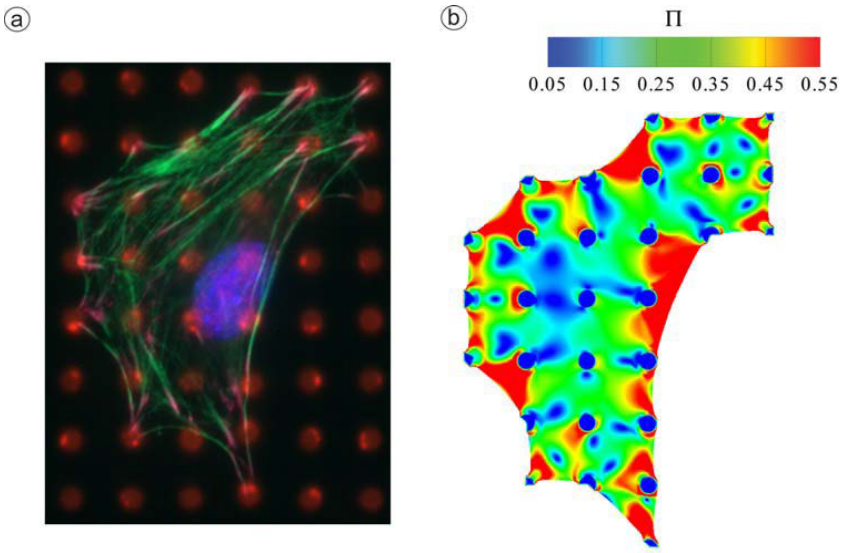


Figure 12. (a) Experimentally observed steady-state actin distributions in a fibroblast cell spread over 29 posts. (b) The corresponding computational results from a simulation of the steady-state actin distribution depicted as a color contour plot of a parameter representing stress fiber concentration.

Cells communicate with their external environment via focal adhesions and generate activation signals that in turn trigger the activity of the intracellular contractile machinery. These signals can be triggered by mechanical loading that gives rise to a

co-operative feedback loop among signaling, focal adhesion formation and cytoskeletal contractility which in turn equilibrates with the applied mechanical loads. We devised a signaling model that couples stress fiber contractility and mechano-sensitive focal adhesion models to complete this above mentioned feedback loop. The signaling model is based on a bio-chemical pathway where IP_3 molecules are generated when focal adhesions grow. These IP_3 molecules diffuse through the cytosol leading to the opening of ion channels that disgorge Ca^{2+} from the endoplasmic reticulum leading to the activation of the actin/myosin contractile machinery. A simple numerical example was developed where a one dimensional cell adhered to a rigid substrate is pulled at one end, and the evolution of the stress fiber activation signal, stress fiber concentrations and focal adhesion distributions are investigated. We demonstrate that while it is sufficient to approximate the activation signal as spatially uniform due to the rapid diffusion of the IP_3 through the cytosol, the level of the activation signal is sensitive to the rate of application of the mechanical loads. This suggests that *ad-hoc* signaling models may not be able to capture the mechanical response of cells to a wide range of mechanical loading events. The setup simulated computationally is illustrated in Figure 13.

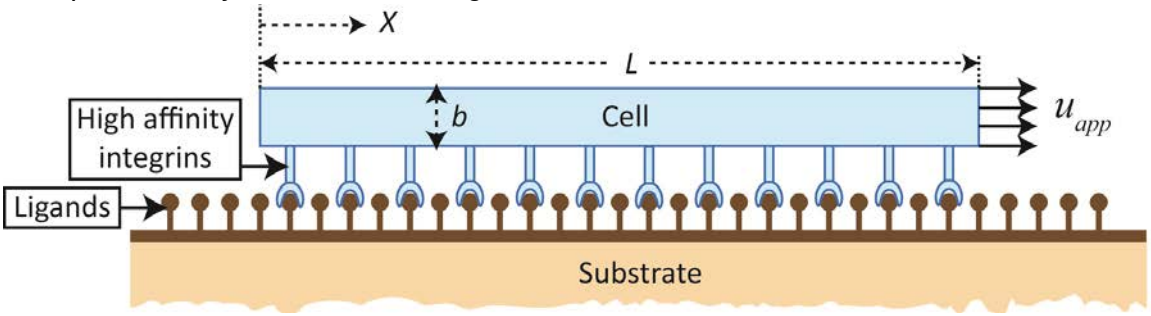


Figure 13. Schematic of the one dimensional cell problem investigated computationally to simulate the interaction of mechanics and signaling. The signals are generated by the application of force to the integrin-ligand bond. The signal generates contractility in the cell, which feeds back to create force at the integrin-ligand bond, thereby stimulating further signaling.

A central thrust of the project is to understand the biophysics of cellular adhesion. Cells from animals adhere to and exert mechanical forces on their surroundings. Cells must control these forces for many biological processes, but how the actions of molecules within a cell are coordinated to regulate the adhesive interaction with the extracellular matrix remains poorly understood. Previously, it was observed that the forces exerted on the extracellular matrix through adhesions varied non-monotonically with changes in velocity of the actin cytoskeleton (Gardel et al. 2008). Now, Dinner, has explored theoretically how measurable subcellular traction stresses depend on the local speed of retrograde actin flow (Li et al., 2010). In the model, forces result from the stretching of molecular complexes in response to the drag from the flow; because these complexes break with extension-dependent kinetics, the flow results in a decrease in their number when sufficiently large (Figure 14). Competition between these two effects naturally gives rise to a clutch-like behavior and a nonmonotonic trend in the measured stresses, consistent with recent data. They used this basic framework to evaluate slip and catch bond mechanisms for integrins; better fits of experimental data are obtained with a catch bond representation. Extension of the model to one comprised of multiple molecular interfaces shifts the peak stress to higher speeds and reveals a phase-transition-like behavior in the dynamics in which slipping between layers can either be distributed uniformly or concentrated at one interface.

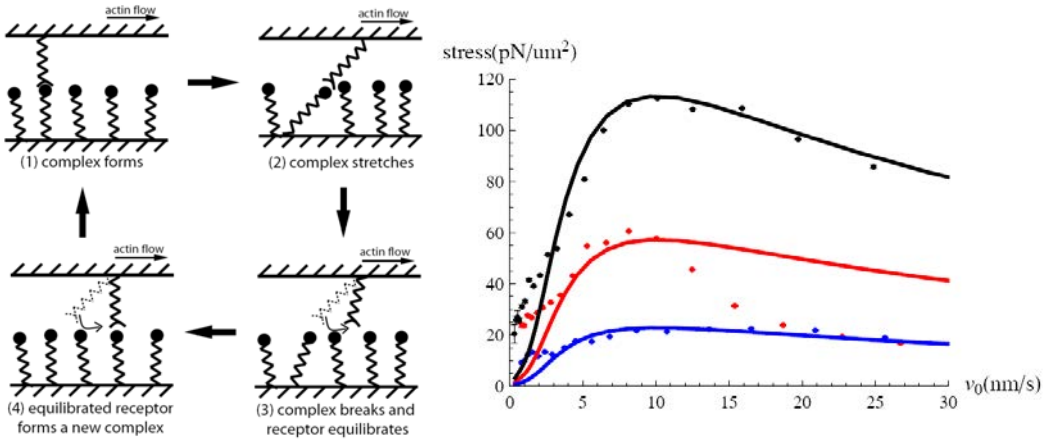


Figure 14. (A) The behaviors of integrin receptors and ligands under the actin flow: integrin receptors capture the ligands and form molecular complexes; complexes stretch elastically and break with extension-dependent kinetics; free receptors equilibrate to their natural length; the cycle repeats. (B)Traction stress for variations in stress fiber density. From top to bottom, k^{u}_{A}/Ak^{A}_{b} = 0.01, 1, 4. Lines are results from the model and points with error bars are experimental data (Fig. 4 of (Gardel et al. 2008)).

Focal adhesions (FAs) are large, multiprotein complexes that provide a mechanical link between the cytoskeletal contractile machinery and the extracellular matrix. They exhibit mechanosensitive properties; they self-assemble upon application of pulling forces and dissociate when these forces are decreased. We rationalize this mechano-sensitivity from thermodynamic considerations and develop a continuum framework in which the cytoskeletal contractile forces generated by stress fibers drive the assembly of the focal adhesion multi-protein complexes. The FA model has three essential features: (i) the low and high affinity integrins co-exist in thermodynamic equilibrium, (ii) the low affinity integrins within the plasma membrane are mobile, and (iii) the contractile forces generated by the stress fibers are in mechanical equilibrium and change the free energies of the integrins. A general two-dimensional framework is presented and the essential features of the model illustrated using one-dimensional examples. Consistent with observations, the coupled stress fiber and FA model predict that: (a) the focal adhesions concentrate around the periphery of the cell; (b) the fraction of the cell covered by focal adhesions increases with decreasing cell size while the total focal adhesion intensity increases with increasing cell size; and (c) the focal adhesion intensity decreases substantially when cell contractility is curtailed. The implementation of the model for cells on complex micro-patterned substrates is illustrated by demonstrating its ability to predict the observed distributions of focal adhesions and stress fibers (Figure 15).

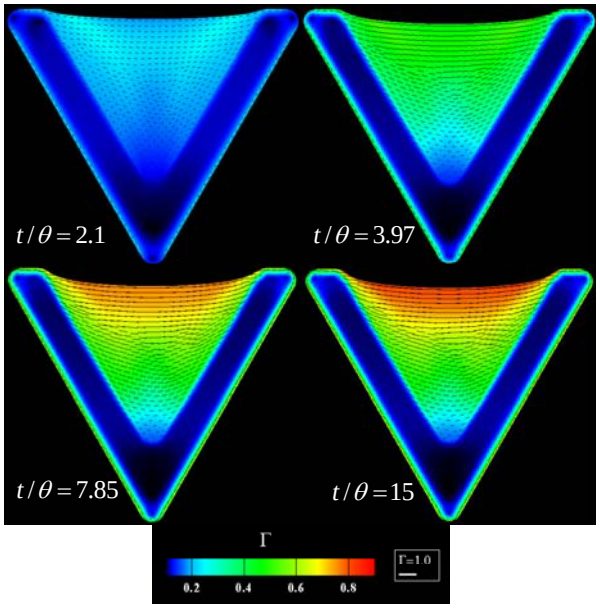


Figure 15. Predictions of the distributions of the stress fibers for a cell on the V-shaped ligand pattern. The predictions are shown at four selected times.